REMARKS/ARGUMENTS

Claims 2-17 and 29-32 were previously cancelled. Claims 23-28 are withdrawn as the result of an earlier restriction requirement. Claim 33 has been added. Claims 1, and 18-22 and 33 are pending in this application.

Claim 1 has been amended to specify that the modified xylanase exhibits increased thermostablity relative to the corresponding natural xylanase. Support for this amendment can be found at page 16, lines 12-13. Furthermore, it is common in the art to compare the activity of a modified enzyme to its naturally-occurring counterpart.

New claim 33 defines a further embodiment of the present invention and defines the basic amino acid of the modified xylanase of claim 1 to one of lysine, arginine, and histidine. Support for this amendment can be found on page 17, lines 16-20, and claim 13 as originally filed.

Rejection under 35 U.S.C. 103

The Examiner has rejected claim 1 under 35 USC 103(a) as being obvious in view of Wakarchuk et al and Sung et al. The Examiner maintains that it would have been obvious to a person of skill in the art to obtain a xylanase with at least one disulfide bond and a basic amino acid at position 162, given the disclosures of Wakarchuk et al. and Sung et al. The Examiner further alleges that Applicant is taking the Sung et al reference out of context as the "natural enzyme" of Sung et al is a naturally thermostable enzyme, while no such requirement is present in claim 1. Applicant respectfully traverses Examiners rejection.

Claim 1 is directed to a modified Family 11 xylanase comprising a basic amino acid at position 162 (determined from sequence alignment of said modified xylanase with *Trichoderma reesei* xylanase II amino acid sequence) and at least one intramolecular disulfide bond. The modified xylanase is further characterized as exhibiting at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 60°C, and exhibiting increased thermostablity relative to the corresponding natural xylanase.

Applicant has shown, for example in figures 3, 4B and 5 of the present application, that xylanase mutants consisting of only a basic amino acid at position 162 (e.g. TrX-162H, with no intramolecular disulfide bond), does not exhibit the characteristic of thermostability relative the corresponding natural xylanase as required in claim 1. However, the combination of the mutation at position 162 with an intramolecular disulfide bond results in significant, synergistic increase (v. additive) in thermostability relative the corresponding natural xylanase (TrX). See for example the activity profiles of TrX-162-DS1 (Figures 3, 4B and 5), TrX-162H-DS4 (Figure 3) or TrX-162H-DS8 (Figure 4B), compared with TrX. Claim 1 of the present application is directed to a xylanase comprising a combination of a mutation at position 162 and an intramolecular disulfide bond.

Sung et al disclose a xylanase mutant, NI-TX1, comprising a basic amino acid at position 162. However, the NI-TX1 mutant does not have a disulfide bridge. At Col 29, lines 44-45 (and with reference to Figure 5) it is stated that:

> *The E.coli-expressed TvX(3-190) showed much less activity at 55 ℃. or above than the natural TrX.*

at line 52-53, that:

> *The mutant NI-TX1, with mutation Q162H, has an activity/temperature profile identical to that of TvX(3-190).*

and, in Col 34, lines 42-43, that:

> *NI-TX1, TvX(3-190), and TrX expressed in E.coli show no improvement in thermostabiulity over the natural TrX.*

There is no teaching or suggestion in Sung et al. to create a modified xylanase having an intramolecular disulfide bridge. Nor is there any teaching or suggestion to create a modified xylanase comprising a mutation at position 162 and an intramolecular disulfide bridge. Furthermore, there is no suggestion in Sung that a disulfide bridge would increase the thermostability of a modified xylanase comprising a basic amino acid at position 162 in accordance with the characteristics defined in claim 1 of the present invention.

Wakarchuk teaches *B. circulans* xylanase mutants with disulfide bridges, and shows varying effects of disulfide bonds on thermostability. For example, the TS1 and TS6 mutants show improved thermophilicity (Figure 4). However, the mutants of Wakarchuk et al do not have a basic amino acid at position 162. There is no suggestion within Wakarchuk of producing a xylanase comprising both a disulfide bridge and a basic amino acid at position 162. As shown in Figure 3 of the present application, xylanase mutants with an intramolecular disulfide bridge (TrX-DS1) exhibit improved thermostability when compared to the native enzyme (TrX). However, a combination of an intramolecular disulfide bridge and a mutation at position 162 results in a significant, synergistic increase in thermostability (e.g. TrX-162H-DS1).

Applicant respectfully maintains that a person of skill in the art would not consider the mutation of position 162 to a basic amino acid in a Family 11 xylanase to be beneficial to thermostability relative to the corresponding natural enzyme, given the teachings of Sung et al. As suggested in the present application on page 16, lines 10-15, the disclosure of Sung et al. may be construed to teach away from mutating the amino acid residue at position 162. Similarly, as shown in figure 3 of the present application, xylanase mutants consisting of a mutation at position 162 (e.g. TrX-162H; no intramolecular disulfide bond), do not exhibit the characteristic of thermostability relative the corresponding natural xylanase (TrX, Figure 3) as required in claim 1, This is to be compared to a modified xylanase enzyme comprising both a mutation at position 162 and an intramolecular disulfide bridge (e.g. TrX-162H-DS1, Figures 3, 4B, 5).

When considered alone, or in combination, Sung et al. and Wakarchuck do not teach or suggest a combination of a modified xylanase comprising a mutation at position 162 and an intramolecular disulfide bridge. Therefore, a person of skill in the art would not have been motivated to combine the teachings of Wakarchuk et al and Sung et al to produce a modified xylanase having improved thermostability compared to the natural enzyme. Nor could they have predicted the synergistic effect of the disulfide bond and the basic amino acid at position 162. Furthermore, while both Sung et al and Wakarchuk et al relate to modification of xylanases to improve thermostability, this does not, by itself, suggest the possibility or desirability of a combination of those references. In re Levitt, 11 U.S.P.Q. 2d 1315 (Fed. Cir. 1989).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited reference itself or in the knowledge generally available to an art worker, to modify the reference so as to arrive at the claimed invention. Second, there must be a reasonable expectation of success, *i.e.*, that the invention would be operable. Finally, the prior art reference must teach or suggest all the claim limitations (M.P.E.P. § 2143). The teaching or suggestion to make the claimed invention and the reasonable expectation of success must both be found in the prior art, not in Applicant's disclosure (M.P.E.P. citing with favor In re Vaeck, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

Applicant submits that there is no suggestion in Wakarchuk and Sung to combine disulfide bonds and a basic amino acid at position 162. One of skill in the art by would not have a reasonable expectation of success in combining Wakarchuk and Sung, as the teaching in Sung suggests that a mutation at position 162 has no beneficial effect thermostability, and this observation is also confirmed in the present disclosure (e.g. Figure 3; activity of TrX-162H v. TrX). Furthermore, it is submitted that neither Wakarchuk nor Sung suggest that the combination of these mutations would lead to increased thermophilicity over the corresponding natural xylanase.

In light of the above comments and amendments, Applicant respectfully requests the withdrawal the rejection under 35 USC 103(a) against claim 1.

It is respectfully submitted that the above-identified application is now in a condition for allowance and favourable reconsideration and prompt allowance of these claims are respectfully requested. Should the Examiner believe that anything further is desirable in order to place the application in better condition for allowance, the Examiner is invited to contact the applicant's undersigned attorney at the telephone number listed below.

Respectfully submitted

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12-30-04 — Date